INFLUENCE OF THE DISTRIBUTION AT BIRTH OF A HEAT SHOCK PROTEINS BOOSTER ON GROWTH AND MORTALITY OF RABBITS BEFORE AND AFTER WEANING

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ABSTRACT

Heat shock proteins (HSPs) are produced by the organism following a stress and are active about 2 hours following the stress. A booster of HSP proteins isolated from the prickly pear *(Opuntia ficus india)*, the *Tex-oe*®, accelerates this production of HSPs which are secreted within 8-10 minutes. This work presents results of the distribution of *Tex-oe*® to the rabbit kits immediately after birth. Four experiment involving 546 litters corresponding to 5416 one-day-old kits were carried out. Half of the one-day old kits received one drop of *Tex-oe*® diluted in a vitamin solution during the hours immediately following their birth. In these conditions, the *Tex-oe*® intake was about 0.01 ml/animal corresponding to 1.6 ml of Tex-oe® for 10 kg live weight. The mortalities and growths of the rabbits were measured from birth to slaughter age at about 70 days. The distribution of this small quantity of *Tex-oe*® immediately after the birth had long term effects for the rabbit and increased the adjusted final weights by 20 g (2273 vs. 2253 g; P=0.088). For mortality, the main observation before and after weaning was a great variability of the results among the experiments: significantly positive effects, significantly negative effects or an absence of effects were observed. The effect of the *Tex-oe*® on the mortality seems to be affected by the pathologic situation of the animals: it was very positive in the first experiment where the dominant pathology was a respiratory one (12.2 vs. 18.5%; P=0.001). In this case, the mortality reduction was particularly important during the finishing stage when the respiratory mortality was the highest. On the contrary, the Tex-*oe*® increased mortality in the 3 other experiments with dominant digestive pathologies (14.0 vs. 11.4% for the control; P=0.034) at the beginning of the fattening period. These different results could probably be explained by an action of the *Tex-oe*® simultaneously protecting the cells against the stress alterations and increasing the feed intake capacity of the animals even after several weeks. This last mechanism may explain the higher growth rate after ingestion of *Tex-oe*®, but may present some inconvenient too in the case of the just weaned rabbit by increasing the digestive problems risks.

Key words: HSPs proteins, Chaperon proteins, Rabbits, Birth stress, Growth performance.

INTRODUCTION

Heat shock proteins (HSPs) also called stress proteins or chaperon proteins are proteins secreted by the cells as a stress feed back (Craig, 1985; David and Grongnet, 2001). They were identified on the thermic stress but act on every type of stress. They protect the other proteins against the stress effects. The HSPs are active 2 hours about after the stress apparition (Feider and Hofman, 1999). A nutritional complement extracted from the prickly pear epicarp (*Opuntia ficus india*) the *Tex-oe*® accelerates the natural production of these HSPs which in these conditions are secreted within 8 to 12 minutes, in place of 120 in normal conditions (Rolland, 2005). Consequently, this product protects the cells against the stress alteration with positive effects in Man (Gutierrez *et al*., 2005), Fish (Powell, 2003) and Chicken. These positive effects were confirmed in the rabbit by distribution of this product at

weaning time (Colin *et al*., 2005). Since some years, the *Tex-Oe* is distributed at the arrival of the chickens in the farm (at 1 and 4 days of age) to alleviate the negative consequences of the adaptation stress. Results are an improvement of growth, feed conversion ratio and viability (Anonymous, 2004; Cruickshank, 2005). The purpose of the present work was to study if a comparable utilisation may be developed in rabbit production by distribution of *Tex-oe*® to one-day–old rabbits, according to a method already described in a preliminary work (Colin *et al*., 2006).

MATERIALS AND METHODS

Distribution of the *Copritex*

The *Copritex* is an alcoholic solution bringing 0.2% of *Tex-oe*®, active principle extracted from the prickly pear epicarp. To be active, this solution should be distributed at a minimum level of 1 ml/10 kg live weight, without negative effect of higher dosages.

In the following experiments, to facilitate the distribution, the *Copritex* was diluted in a vitamin solution (25% of *Copritex* – 75% of vitamin and amino acid solution). One drop of this mix was individually distributed to the just born rabbits during the hours following immediately their birth. In these conditions, the *Tex-oe*® intake was about 0.01 ml/animal corresponding to 1.6 ml of Tex-oe® for 10 kg live weight.

General presentation of the experiments

Four experiments involving 546 litters corresponding to 5416 one-day-old rabbits have been carried out between April 2006 and October 2006 (Table 1). These litters come from 3 different maternity buildings of the same experimental farm (Earl 3L).

Table 1: Rabbits involved in the 4 experiments

Periods / Criteria	Treatment	Experiments				N° per treatment	Total N° involved
		1	2	3	4		
At birth							
Date of birth in 2006		*9 April*	*22 May*	*3 July*	*14 August*		
N° litters	Control	72	85	117	68	342	546
	Copritex	27	79	28	70	204	
N° one day old kits	Control	738	838	1 159	637	3 372	5 416
	Copritex	283	792	282	687	2 044	
Weaning age in days		*35*	*37*	*38*	*36*		
After weaning							
N° cages	Control	63	123	47	144	377	754
	Copritex	59	125	49	144	377	
N° rabbits	Control	441	615	259	716	2 031	4 025
	Copritex	409	625	248	712	1 994	
Age at "middle" control, in days		*54*	*54*	*55*	*55*		
Final age in days		*75*	*69*	*69*	*69*		

At one day, the rabbits of the Copritex treatment were treated according the above described method. Mortality was registered and the kits were weighted at 15 days of age (except in the second experiment). Kits were weaned between 35 and 38 days according to the experiment. At weaning, rabbits were divided in two groups homogeneous according to maternity origin, weaning weight and treatment in maternity (Control and Copritex). Rabbits were weighed per cage at weaning, in the middle of the fattening period (54-55 days of age) and at the end of the experiment. For each stage of control, weights were expressed as individual rabbit weight taking account of the number of rabbits alive per cage. Mortality was controlled every day and some rabbits were autopsied. The apparent cause of death was registered as digestive trouble (diarrhea and/or digestive tract disorder at autopsy), as respiratory trouble (important coryza and/or pulmonary disorder at autopsy, without digestive trouble) or as undetermined or multiple cause.

Maternity and fattening buildings were with dynamic ventilation. Maternity buildings were without windows with a lighting program of 16 light/8 hours dark. The fattening ones were with natural light through opening in the walls. During this fattening period, rabbits were 7/cage in the first experiment and 5 to 6/cage in the 3 other ones. Rabbits received *ad libitum* a feed with 18% proteins and 14% crude fiber in maternity and 15% proteins and 14% crude fiber after weaning. No medical treatment was carried out, neither through the feed, nor through drinking water because the experimental farm was antibiotic free since 4 years. The vitamin and amino acid solution used to dilute the Copritex brought the following quantities of nutrients per liter: Vitamin B_1: 7500 mg, Vitamin B_2: 3000 mg, Vitamin B_6: 3000 mg, Vitamin B_{12}: 15 mg, Biotin: 7.5 mg, Pantothenic acid: 15500 mg, L-Lysin: 2250 mg, DL methionin: 375 mg, L-Threonin: 375 mg.

Statistical analysis

The rabbit weights until weaning were studied by variance analysis according to the experimental design with 2 treatments x 3 maternity buildings x 4 successive experiments and interactions (Dagnelie, 1969). The weights and growth rate (ADG) during the fattening period were studied with the same statistical design but by covariance analysis (covariate: weaning weight, adjustment of means by the least squares method) in order to eliminate the possible effect of difference on weaning weight. Mortalities before and after weaning were also studied by variance analysis giving the value 1 to every dead rabbit and 0 to rabbits alive at the end of the period taken in consideration. For the pre-weaning period, the analyzed data correspond to litters with at least one rabbit alive at weaning.

RESULTS AND DISCUSSION

Maternity

During the 4 experiments, the mortality remained at a level comprised between 6.1 and 11,5% (Table 2) without symptom of a specific pathology. A positive effect of the Copritex treatment with statistical tendency (P=0.065) was observed in the second experiment (where the mortality was the highest) and a negative effect in the fourth (P=0.016). Summarizing the 4 experiments, there was no significant effect of the treatment with Copritex, nor interaction between treatment and experiment. The mortalities between maternity buildings were significantly different but the interaction with treatments was not (P>0.10).

Table 2: Birth to weaning mortality expressed as percentage of rabbits born alive in litters with at least one kit alive at weaning

Experiment		1	2	3	4	Overall mean	Residual mean std
Treatment	Control	8.26	11.45	8.54	6.12	8.75	0.54%
	Copritex	8.12	9.46	7.09	9.46	8.95	
Within experiment probability ¨P		*0.367*	*0.065*	*0.228*	*0.016*	*0.901*	

Weight of 15 days old kits was not affected by the treatment of Copritex (281 ± 36 g on average). At weaning, average kit's weight was negatively affected by Copritex treatment in experiment 2, and a tendency to the opposite effect (P=0.09) was observed in the experiment 4 (Table 3). Altogether, the treatment of day old kits with Copritex has no significant effect on kit's weight until weaning in a constant manner.

Table 3: Average individual weight of young rabbits at weaning

Experiments		1	2	3	4	Overall mean	Residual mean std
Average weight at weaning (g)	Control	930	1083	990	950	995	3.9
	Copritex	936	1052	994	934	981	
Within experiment probability ¨P		*0.305*	*0.004*	*0.592*	*0.094*	*0.110*	

Fattening period

On a general point of view, the mortality was associated to respiratory symptoms in the first experiment and to the classically digestive symptoms generally observed in the EARL 3L (Colin *et al*., 2007) in the 3 other experiments. The mortality during the fattening period was highly significantly improved with Copritex in the first experiment due to a strong reduction of the mortality during the finishing period (Figure 1, Table 4). On the contrary, mortality was significantly increased with Copritex in the 4th experiment, mainly during the beginning of the fattening. The interaction treatment–experiment was highly significant, but altogether Copritex had no average significant effect on mortality (Figure 1).

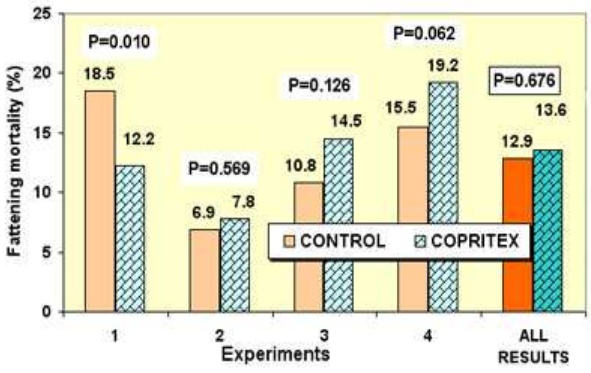

Figure 1: Average mortality from weaning to slaughter age

Nevertheless, if the 3 experiments where only digestive troubles were observed, are considered together Copritex treatment appears to increase the overall mortality (14.0 vs.11.4%; P=0.034) with an increase mainly during the weeks following weaning (P=0.012), but with no significant effect during the last part of the fattening period (P=0.507).

Table 4: Mortalities observed during the 2 parts of the fattening period

Experiments		1	2	3	4	Overall mean	Residual mean std
Mortality 1st part %	Control	6.1	4.9	3.5	5.3	5.12	
	Copritex	4.2	3.5	7.2	9.8	6.37	0.52
Within experiment probability ¨P		*0.197*	*0.233*	*0.058*	*0.001*	*0.115*	
Mortality 2nd part %	Control	13.2	2.2	7.6	10.3	8.30	
	Copritex	8.4	4.4	7.8	10.4	7.77	0.62
Within experiment probability ¨P		*0.027*	*0.031*	*0.926*	*0.846*	*0.474*	

Average daily growth was improved by Copritex initial administration in the experiment 1, mainly during the weeks following weaning (Table 5). In each of the 3 other experiments, the effect of Copritex was not significant. Nevertheless, when the 4 experiments were considered together, a significant improvement of rabbit's live weight was observed in the middle of the fattening period for the Copritex group (+18 g).

Table 5: Weight performance (Least square means adjusted for a common weaning [initial] weight of 988 g)

Experiments		1	2	3	4	Overall mean	Residual mean std
Mid-fattening live weight (g)	Control	1475	1801	1684	1686	1667	
	Copritex	1543	1799	1678	1701	1685	10.3
Within experiment probability ¨P		*0.002*	*0.862*	*0.708*	*0.248*	*0.018*	
Final live weight (g)	Control	2263	2296	2175	2280	2253	
	Copritex	2336	2281	2165	2291	2273	16.7
Within experiment probability ¨P		*0.093*	*0.484*	*0.671*	*0.569*	*0.088*	
Average fattening growth rate (g/d)	Control	33.2	37.9	38.2	40.5	37.41	
	Copritex	34.8	38.4	37.8	40.9	38.03	0.49
Within experiment probability		*0.093*	*0.484*	*0.671*	*0.570*	*0.112*	

This advantage continue to be observed at the end of the fattening period, as a statistical tendency (+20 g; P=0.088). If the interaction between treatment and number of experiment was significant for the mid-period live weight (P=0.011), this interaction was not significant for the final weight (P=0.230) nor for the average daily gain (P=0.345).

GENERAL DISCUSSION

These experiments demonstrate that a distribution of a small quantity of *Tex-oe*® during the first hours of life of a rabbit has long term effects as for the chicken (Anonym, 2004; Cruickshank, 2005): a distribution of about 0.01 ml/animal tends to increase the adjusted final weights by 20 g, value to compare to the 50 g improvement previously observed with a distribution of *Tex-oe* ® at weaning time (Colin *et al*., 2005). These beneficial effects take place at the beginning of the growing-fattening period because no differences were observed when the final weight was adjusted by covariance on the intermediary one. For mortality, the main observation before and after weaning was an important variability of the results among the experiments: it can be observed significantly positive effects, significantly negative effects or an absence of effects. These results are in agreement with our previous observations for the fattening period (Colin *et al.*, 2005) but are lightly different in maternity (Colin *et al*., 2006). The effect of the *Tex-oe*® on mortality seems affected by the pathologic situation of the animals: the effect was very positive in the first experiment with a dominant respiratory pathology but it was more or less negative in the 3 other ones with predominant digestive troubles. These results can probably be explained by an action of the *Tex-oe*® simultaneously protecting the cells against the stress alterations (Craig, 1985) and increasing the feed intake capacity of the rabbits even after several weeks as it is observed for other types of animals (Powel, 2003; Anonymous, 2004). The hypothesis of intervention of such a mechanism is coherent with the higher growth observed after ingestion of *Tex-oe*® but which may present some inconvenient too in the case of the weaning rabbit by increasing the digestive problems risk associated with a too high ingestion level (Gidenne *et al.*, 2007). In conclusion, this work confirms that the *Tex-oe*® presents long term effects when distributed immediately after a stress such that of birth, but the variability of the obtained results makes necessary other theoretical studies before generalizing its utilization in rabbit production.

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